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ABSTRACT

Breast cancer development typified by the overexpression of growth factors and growth factor receptors, expression of cell cycle markers such as cyclin D1 and c-myc, expression of chemokines such as RANTES, and development of resistance to cancer therapies. We and others have provided evidence that the transcription factor NF-kappaB and associated activities are expressed/activated in human breast cancer. Specifically we found that the NF-kB2/p52 NF-kappaB subunit and Bcl-3 are expressed in a significant number of breast tumors. Our goals were to: (i) identify genes regulated by Her-2/neu and Bcl-3 that may be relevant to the progression of the disease, (ii) determine the mechanism whereby Bcl-3 blocks cancer-therapy induce apoptosis, and (iii) determine if Bcl-3 and the associated NF-kappaB subunit p52 are required for the development of experimental breast tumors in animal models. These goals may provide significant new insight into breast cancer progression and treatment.

Table of Contents

Cover.....

SF 298.....

Table of Contents..... 3

Introduction..... 4

Body..... 4

Key Research Accomplishments..... 5

Reportable Outcomes..... 5

Conclusions..... 6

References..... 6

Appendix I 7-12

Appendix II (Methods and Figure Legends for Appendix I) 13-20

INTRODUCTION:

Development and progression of breast cancer is characterized by dysregulation of growth factors, growth factor receptors, expression of cycle cell markers including cyclin D1 and c-myc, expression of chemokines such as RANTES, and development of resistance to cancer therapies. This type of dysregulation can be explained in a global sense through the inappropriate activation of transcription factors. We and others have provided evidence that NF-κB activity is activated in breast cancer cell lines and in a majority of human breast tumors (for example, see Cogswell et al., 2000; Romieu-Mourez et al., 2003). Recently, Hung and colleagues provided evidence that the kinase that regulates NF-κB (IKKβ) is active in breast cancer and is involved in the inactivation of a pro-apoptotic transcription factor Foxo3a (Hu et al., 2004). Our previous analysis in human breast tumor tissues is that p52/NF-κB2 (a non-classic form of NF-κB) and Bcl-3, an IκB homologue, are upregulated in a number of breast tumors (Cogswell et al., 2000). It was also reported that Bcl-3 is upregulated in breast cancer cells following withdrawal of estrogen, leading to growth and hormone independence (Pratt et al., 2003). These findings relate to our previous publication (Westerheide et al., 2000) that Bcl-3 can directly regulate cyclin D1 gene expression (which is known to be upregulated in a majority of breast tumors). Our goals are to understand the biological roles of the NF-κB regulatory system (including Bcl-3) in breast cancer as related to development of this disease as well as in controlling cancer therapy resistance.

BODY:

The Specific Aims/Statement of Work indicate the following priorities: (year 1) identify genes and regulatory processes regulated by Bcl-3 and by Her-2/Neu, (year 2) determine mechanisms associated with the ability of Bcl-3 to alter responses to chemotherapies and radiation, and (year 3) determine if Bcl-3 and/or p52 are required for the development of experimental breast tumors using animal models.

In year one we had proposed to begin to identify genes and regulatory processes controlled by Bcl-3 and induced by Her-2/Neu. In year two, we had proposed to determine the mechanisms associated with the ability of Bcl-3 to alter responses to chemotherapy. We have made significant progress on both of these aims. We generated MCF-7 breast cancer cells that overexpress Bcl-3 and found the following: (i) these cells are highly resistant to UV-induced and chemotherapy-induced cell death and (ii) these cells exhibit a blunted p53 response (see Appendix I).

These data suggest that breast cancer cells that express Bcl-3 will be resistant to the induction of apoptosis by cancer therapy, at least partly through the ability to suppress p53 function. Correspondingly, we analyzed Bcl-3 null cells, and found that Bcl-3 expression functions normally inhibits UV-induced and chemotherapy-induced p53 activation. This mechanism appears to require the ability of Bcl-3 to induce Mdm2 function (see Appendix I). Thus, the ability of Bcl-3 to suppress p53 induction appears to involve the Bcl-3 dependent activation of Mdm2 gene expression.

Additionally, although we were unable initially to maintain the Her-2/Neu-expressing H16N2 cells, we have now established those cells for subsequent experiments relating Her-2/Neu expression with Bcl-3 activity. We have initiated a microarray study to identify genes regulated by Her-2/Neu in an NF-κB-dependent manner. This array has been done once and we are currently repeating the array to confirm the findings. The initial results demonstrate an unexpected set of genes regulated (both positively and negatively) by Her-2/Neu in an NF-κB-dependent manner. These genes are likely to strongly contribute to Her-2/Neu-mediated breast cancer. Curiously, we have found that Her-2/Neu expression suppresses Bcl-3 expression. Although, we have received the Bcl-3 null mice (which will allow us to begin our animal experimentation) we may have to wait and their initiation in order confirm the downregulation of Bcl-3 in Her-2/Neu-expressing cells (which would suggest that the original animal model proposed might not be relevant).

KEY RESEARCH ACCOMPLISHMENTS:

-Generation of Bcl-3 expressing MCF-7 breast cancer cell lines for studies on Bcl-3-dependent gene expression and control of chemoresistance (Fig. 1A, Appendix I).
-Demonstration that expression of Bcl-3 strongly blocked the ability of DNA damaging stimuli (including chemotherapy) to induce cell death. The data indicate that one function of Bcl-3 expression in breast cancer is to block cancer therapy-induced cell death (Fig. 1B, Appendix I).
-Demonstration that the ability of p53 to be induced by chemotherapy is strongly suppressed in Bcl-3-expressing MCF-7 breast cancer cells (Fig. 2A, Appendix I).
-Evidence that Bcl-3 expression blocks the induction of p53-dependent genes (Fig. 2C, Appendix I).
-Experimental evidence that knockdown of expression of Bcl-3 leads to the ability of p53 to be induced by DNA damaging agents (Fig. 3A, Appendix I).
-Demonstration that the role of Bcl-3, endogenously, is to limit the activation of p53 and to limit apoptosis (Fig. 3C, Appendix I).
-Demonstration that expression of Bcl-3 is correlated with expression of Mdm2, the inhibitor of p53. Expression of Bcl-3 transiently in several cell types lead to the upregulation of Mdm2 gene expression (Fig. 4, Appendix I). .
-Obtained Bcl-3 null animals and generated primary fibroblasts that are Bcl-3 -/- for use in the analysis of the role of Bcl-3 in responses to DNA damage and apoptosis (Fig. 5, Appendix I).
-Bcl-3 null cells are inhibited in their ability to induce Mdm2 gene expression, and correspondingly exhibit elevated p53 activation response (Fig. 5B, Appendix I).
-Obtained the compound RITA which suppresses p53 and Mdm2 interaction. Utilized this compound to show that the ability of Bcl-3 to suppress p53 activation is controlled at the level of Mdm2/p53 interaction, suggesting that the ability of Bcl-3 to upregulate Mdm2 controls its ability to block p53 activation (Fig. 6, Appendix I).
-Performed an initial microarray analysis to identify NF-κB-regulated genes induced in response to expression of Her2/Neu (ErbB2). While this experiment needs to be repeated, NF-κB appears to control the expression of genes involved in promoting cell proliferation and regulating apoptosis (data not shown, until this work is repeated).

REPORTABLE OUTCOMES:

We now show (Kashatus et al., "Expression of the Bcl-3 Oncogene Suppresses p53 Activation", submitted to *Cancer Cell*) that Bcl-3 expression in breast tumor cells strongly suppresses cell

death induced by chemotherapy. As described above, this appears to be regulated through the ability of Bcl-3 to block p53 activation through the upregulation of the p53 inhibitor Hdm2/mdm2. This is a key finding regarding the function of Bcl-3 and relevant to understanding why breast cancer may be resistant to cancer therapy treatment.

CONCLUSIONS:

Bcl-3, which is described as an oncoprotein in certain leukemias and lymphomas, is expressed in breast cancer animal models and in human breast tumors. Our present data indicate the very interesting possibility that Bcl-3 controls p53 tumor suppressor function in a negative manner while functioning to promote cell proliferation through upregulation of Cyclin D1 (often overexpressed in breast cancer). The data would suggest that breast tumors with Bcl-3 expression are actually inhibited for the tumor suppressor function of p53 via the potential upregulation of Mdm2. The data may also explain why Bcl-3-expressing tumors are chemo- or radioresistant and raise the potential of inhibiting Bcl-3 as a new therapeutic direction. The data also raise the interesting possibility that Mdm2 has functions separate from inhibiting p53 and that Bcl-3 promotes this process.

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Hu, M.C., D.F. Lee, W. Xia, L.Golfman, F. Yang, J. Yang, Y. Zou, S. Bao, N. Hanada, H. Saso, R. Kobayashi, and M.C. Hung. 2004. IKK promotes tumorigenesis through inhibition of Forkhead Foxo3a. Cell 117, 225-237.

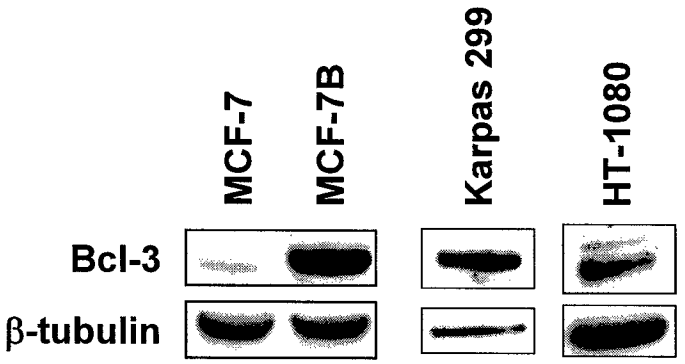
Pratt, M.A., T. Bishop, D. White, G. Yasvinski, M. Menard, M. Niu, and R. Clarke. 2003. Estrogen withdrawal-induced NF-kB activity and Bcl-3 expression in breast cancer cells: roles in growth and hormone independence. Mol. Cell. Biol. 23, 6887-6900.

Romieu-Mourez, R., D. Kim, S.Shin, E. Demico, D. Landesman-Bollag, D. Seldin, R. Cardiff, and G. Sonenshein. 2003. Mouse mammary tumor virus c-rel transgenic mice develop breast tumors. Mol. Cell. Biol. 23, 5738-5754.

Westerheide, S., M. Mayo, V. Anest, J. Hanson, and A. Baldwin. 2001. The putative oncoprotein Bcl-3 induces cyclin D1 to induce G1 transition. Mol. Cell. Biol. 21, 8428-8436.

Figure 1

A)


MCF-7
MCF-7B
Karpas 299
HT-1080
Bcl-3
β-tubulin

B)

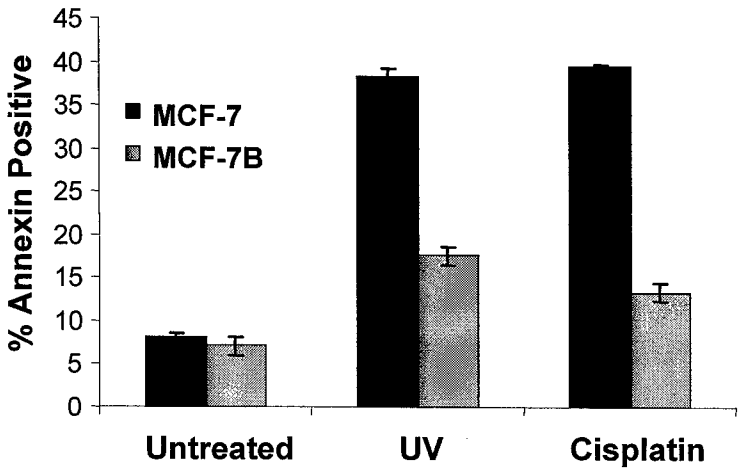
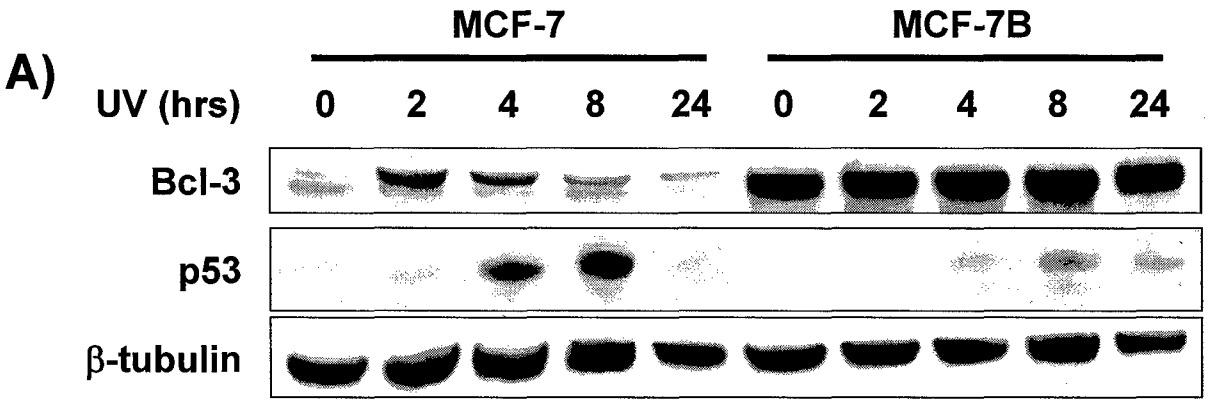
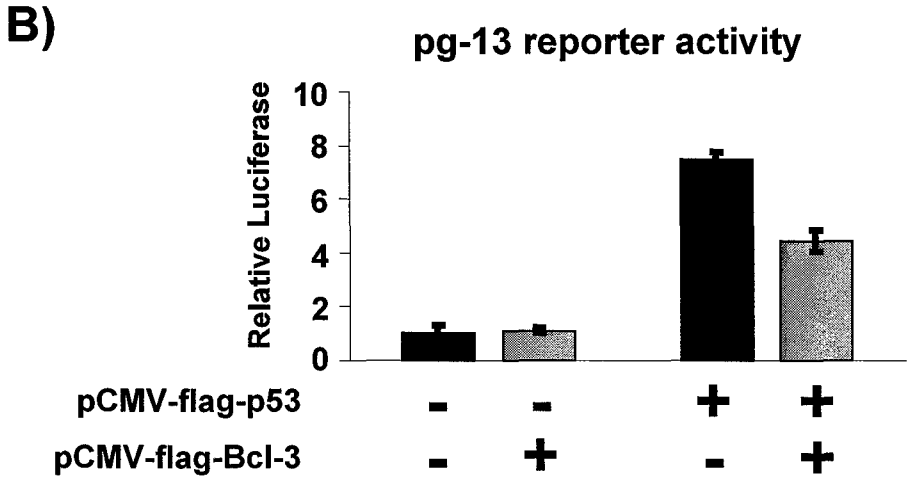

% Annexin Positive
45
40
35
30
25
20
15
10
5
0
MCF-7
MCF-7B
Untreated
UV
Cisplatin

Figure 2


A)
MCF-7
MCF-7B
UV (hrs)
0 2 4 8 24 0 2 4 8 24
Bcl-3
p53
β-tubulin


B)
pg-13 reporter activity
Relative Luciferase
10
8
6
4
2
0
pCMV-flag-p53
pCMV-flag-Bcl-3
- - + +
- + - +

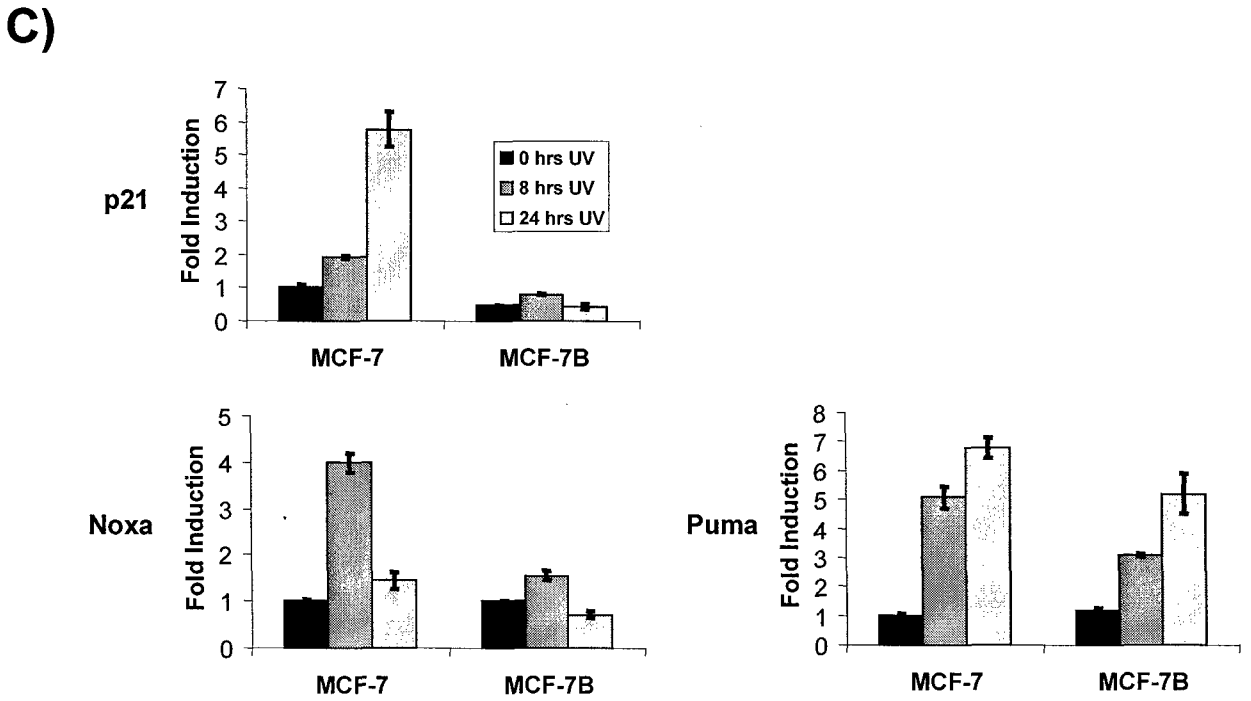

C)
p21
Fold Induction
0 hrs UV
8 hrs UV
24 hrs UV
MCF-7
MCF-7B
Noxa
Fold Induction
MCF-7
MCF-7B
Puma
Fold Induction
MCF-7
MCF-7B

Figure 3

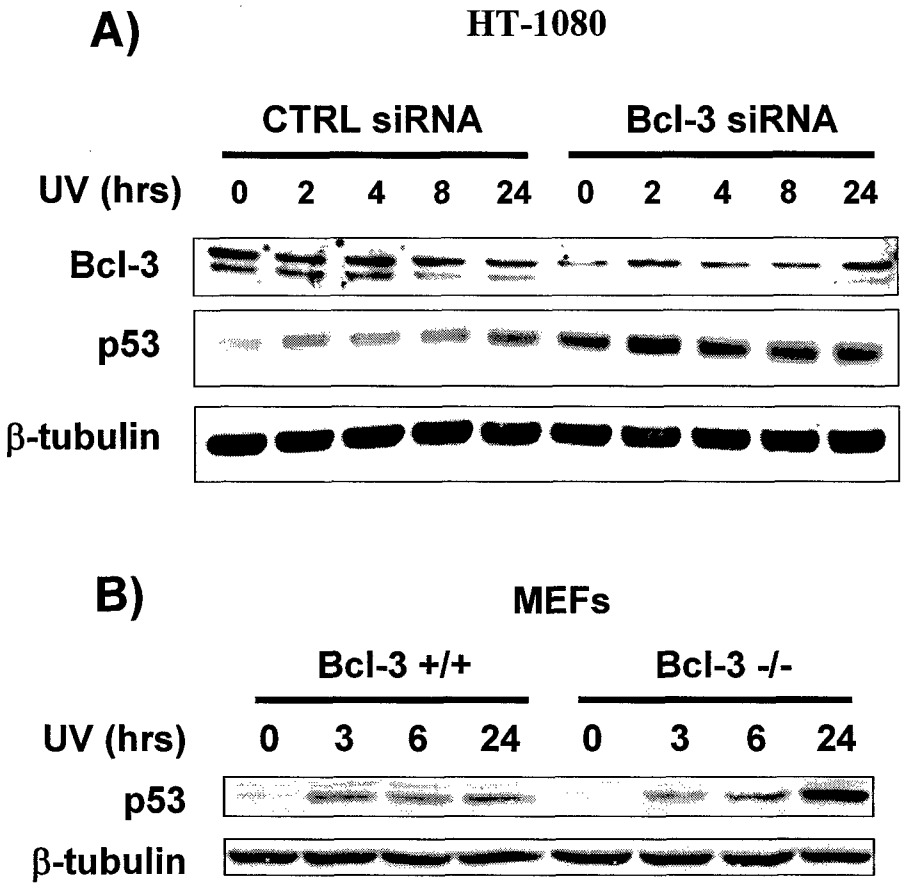

A)
HT-1080
CTRL siRNA
Bcl-3 siRNA
UV (hrs)
0 2 4 8 24 0 2 4 8 24
Bcl-3
p53
β-tubulin
B)
MEFs
Bcl-3 +/+
Bcl-3 -/-
UV (hrs)
0 3 6 24 0 3 6 24
p53
β-tubulin
C)
UV-induced apoptosis
% Annexin Positive
30
25
20
15
10
5
0
Bcl-3 +/+
Bcl-3 -/-
Untreated
UV

Figure 4

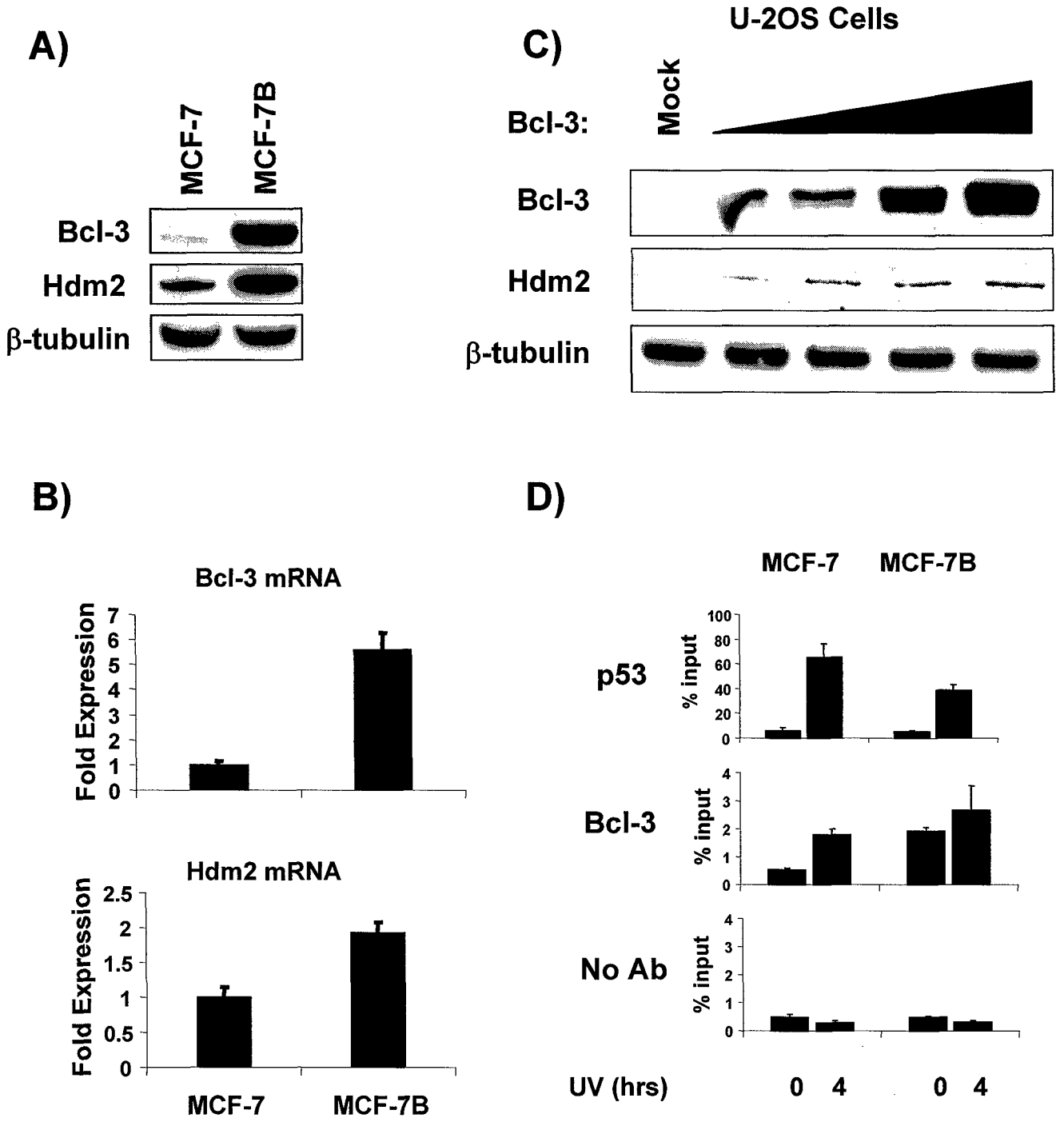
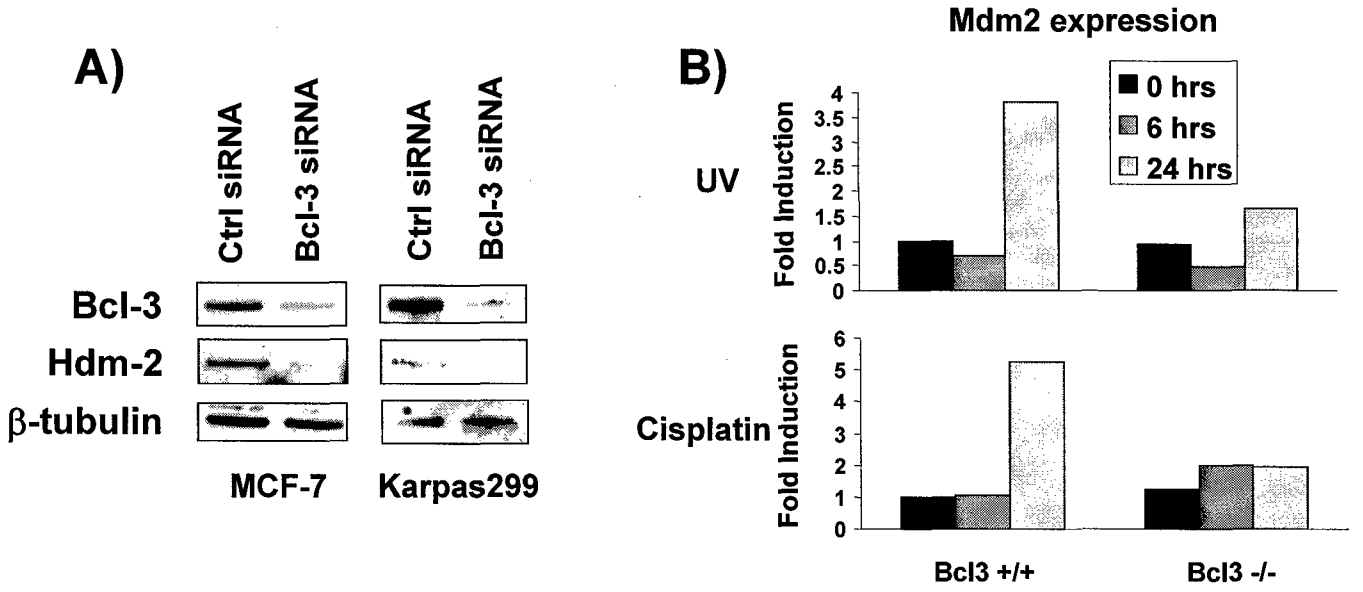
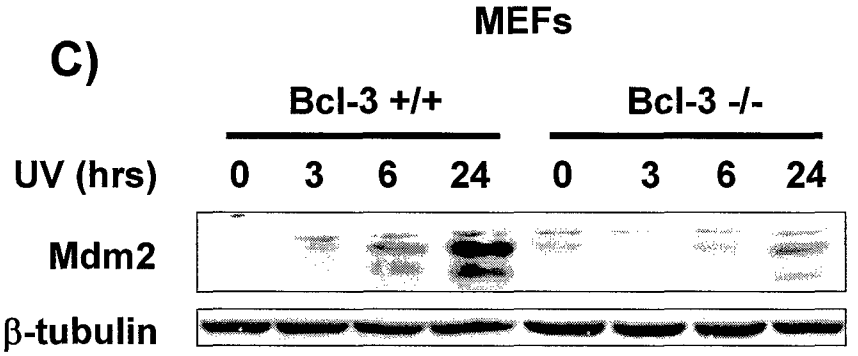

A)
MCF-7
MCF-7B
Bcl-3
Hdm2
β-tubulin
C)
U-2OS Cells
Mock
Bcl-3:
Bcl-3
Hdm2
β-tubulin
B)
Bcl-3 mRNA
Fold Expression
0
1
2
3
4
5
6
7
Hdm2 mRNA
Fold Expression
0
0.5
1
1.5
2
2.5
MCF-7
MCF-7B
D)
MCF-7
MCF-7B
p53
% input
0
20
40
60
80
100
Bcl-3
% input
0
1
2
3
4
No Ab
% input
0
1
2
3
4
UV (hrs)
0
4
0
4

Figure 5


A)
Ctrl siRNA
Bcl-3 siRNA
Ctrl siRNA
Bcl-3 siRNA
Bcl-3
Hdm-2
β-tubulin
MCF-7
Karpas299
B)
Mdm2 expression
UV
Fold Induction
0 hrs
6 hrs
24 hrs
Cisplatin
Fold Induction
Bcl3 +/+
Bcl3 -/-


C)
MEFs
Bcl-3 +/+
Bcl-3 -/-
UV (hrs)
0 3 6 24 0 3 6 24
Mdm2
β-tubulin

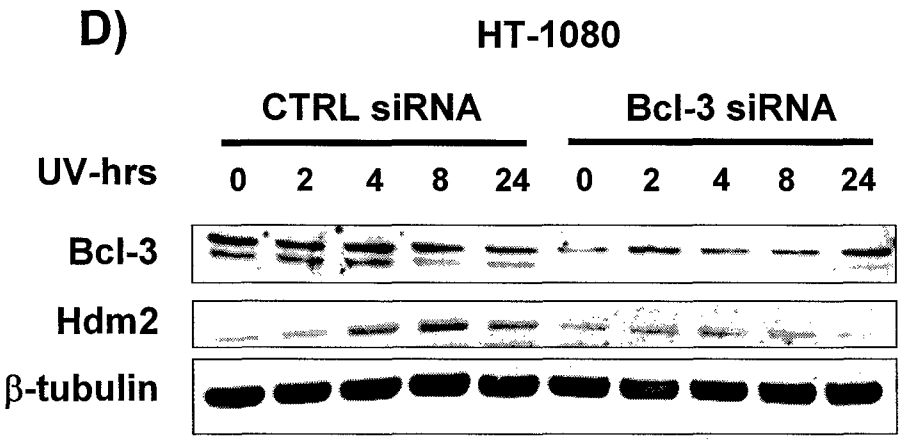
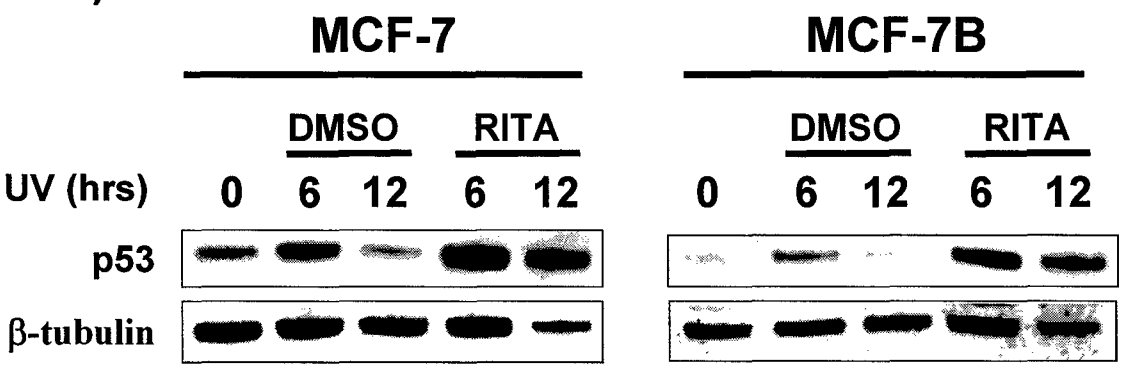
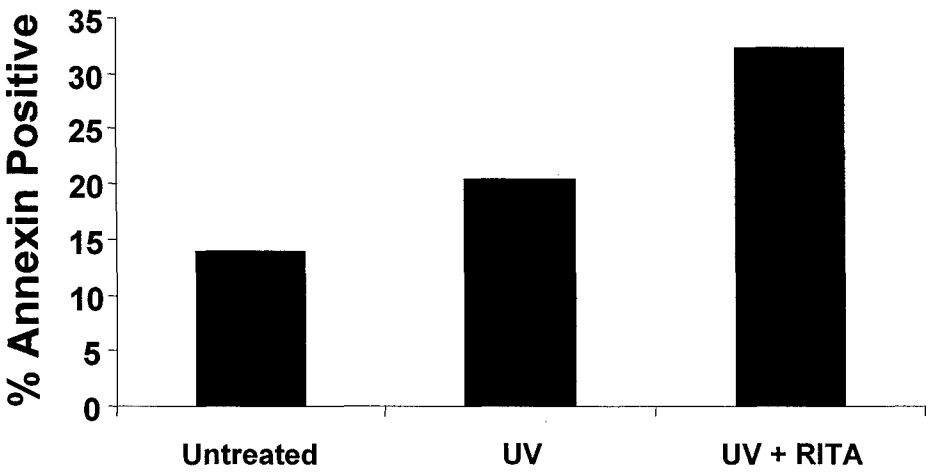

D)
HT-1080
CTRL siRNA
Bcl-3 siRNA
UV-hrs
0 2 4 8 24 0 2 4 8 24
Bcl-3
Hdm2
β-tubulin

Figure 6

A)


MCF-7
MCF-7B
DMSO
RITA
DMSO
RITA
UV (hrs)
0
6
12
6
12
0
6
12
6
12
p53
β-tubulin

B)


% Annexin Positive
35
30
25
20
15
10
5
0
Untreated
UV
UV + RITA

EXPERIMENTAL PROCEDURES:

Cell Culture

Primary murine embryo fibroblasts were isolated from day 13 embryos and grown in DMEM supplemented with 10% FBS (Sigma) and 1X penicillin/streptomycin (Gibco). MCF-7 cells (ATCC) were grown in MEMα supplemented with 10% FBS, 10 ug/ml Insulin (Gibco), 1mM Sodium Pyruvate (Gibco) and 1X penicillin/streptomycin. Karpas 299 cells (DSMZ, Braunschweig, Germany) were grown in RPMI supplemented with 10% fetal bovine serum and 1X penicillin/streptomycin. HT1080 cells (ATCC) were grown in DMEM supplemented with 10% FBS and 1X penicillin/streptomycin. U-2OS cells (ATCC) were grown in McCoys 5A supplemented with 15% fetal bovine serum and 1X penicillin/streptomycin. MCF-7B cells were generated by transfecting the expression construct pFlag-Bcl-3 into MCF-7 cells. Stable clones were generated in medium containing 1 μg/ml puromycin (Sigma). Clones were verified by Western blotting with a Bcl-3-specific antibody. Cisplatin (Sigma) was resuspended in DMSO and stored at –20°C. RITA was resuspended in DMSO to a concentration of 10mM and stored in the dark at –20°C. For UV treatments, cells were placed under a UV lamp and dosage was measured with a UV-X radiometer (Ultra Violet Products, Upland, CA).

Antibodies

For western blots and ChIP analysis we used antibodies against p53 (FL393, Santa Cruz), Bcl-3 (Upstate), Hdm2 (Ab-1, Calbiochem), mdm2 (2A10, from A. Levine) and β-tubulin (H-235, Santa Cruz). HRP-conjugated anti-mouse and anti-rabbit IgG secondary antibodies were from Promega.

Western Blot

Typically, cells were plated in a 100 mm dish and treated with the indicated dose of UV. After the indicated timepoints, cells were wash with PBS and lysed in modified RIPA buffer (1% NP40, 20mM Tris, 137 mM NaCl, 10% glycerol, 2mM EDTA, 2 μg/mL aprotinin, 2 μg/mL leupeptin, 1 mM PMSF). 10-15 μg protein were loaded onto 4-12% Bis-Tris gels (Invitrogen). After electrophoresis, gels were transferred to nitrocellulose (Biorad) and blocked for 1 hr in TBS containing 0.5% Tween-20, 4% milk and 1% BSA. Primary and secondary antibody incubations were performed in blocking buffer at 4°C (primary) or room temp (secondary).

Apoptosis Detection

Cells were plated in 100mm dishes and treated with the indicated dose of UV or cisplatin. At the indicated timepoints, cells were washed with PBS and collected by trypsinization. Cells were washed again in PBS and resuspended in 100 μL annexin binding buffer (10mM HEPES, 140mM NaCl, 2.5mM $CaCl_2$, pH 7.4)) containing 5 μL Alexa-488 conjugated Annexin-V (Molecular Probes) plus 1 μg/ml propidium iodide (Sigma). Cells were incubated for 15 minutes at room temperature and then analyzed on a Facscalibur (Becton Dickinson). Apoptotic cells were measured as positive for Annexin-V staining but negative for propidium iodide.

Plasmid Constructs

pCMV2-flag Bcl-3 (Westerheide et al., 2001) pCMV-flag-p53 (Zhang et al., 1998) and pg-13-luciferase (el-Deiry et al., 1993) were described previously.

ChIP Assay

ChIP analysis was performed using a chromatin immunoprecipitation kit (Upstate Biotechnology) and a modified version of the manufacturers protocol. Following the indicated treatment, cells were fixed for 5 minutes in 1% formaldehyde, washed with PBS and lysed for 10 min in lysis buffer. Chromatin was sheared by sonication to an average size of approximately 1 kilobase and pre-cleared for 2 h at 4 °C with salmon sperm DNA-saturated protein G Sepharose beads. Chromatin solutions were precipitated overnight at 4°C using 10 µl of the indicated antibodies. Immune complexes were collected with salmon sperm DNA-saturated protein G Sepharose beads for 1 h and washed extensively following the manufacturer's protocol. Input and immunoprecipitated chromatin were incubated at 65 °C overnight to reverse crosslinks. After proteinase K digestion, DNA was extracted with phenol/chloroform and precipitated with ethanol. Precipitated DNAs were analysed by Real Time PCR on an ABI 7100 using SYBR green master mix (ABI). Each sample was normalized to input using the $2^{-\Delta\Delta C(T)}$ method (Livak and Schmittgen, 2001). Hdm2 P2 promoter specific primers used were: 5'-GAGGTCCGGATGATCGCAGG-3' and 5'-GTGGCGTGCGTCCGTGCCCA-3'.

Luciferase Assays

Typically, cells were plated in 24-well dishes and allowed to grow to ~70% confluency. Cells were transfected with the indicated plasmids using polyfect reagent (Qiagen) following the manufacturers protocol. 48 hours post-transfection, extracts were prepared using the Dual Luciferase Assay System (Promega, Madison, WI) following the manufacturers protocol and luciferase activity was measured on an LMax luminometer (Molecular Devices, Sunnyvale, CA).

Real Time PCR

Cells were plated in 100 mm dishes and treated with the indicated doses of UV or cisplatin. At the indicated timepoints, cells were washed in PBS, lysed in Trizol Reagent (Invitrogen) and RNA was collected following the manufacturers protocol. cDNA was generated using the M-MLV reverse transcriptase kit (Invitrogen) and quantitative PCR was performed on an ABI Prism 7000 (Applied Biosystems, Foster City, CA) using gene specific TAQman primer/probe sets (Applied Biosystems).

RNAi

Synthetic dsRNA oligonucleotides targeting Bcl-3 were purchased from Xeragon. The targeted sequence is 5'-AATGGTCTTCTCTCCGCATCA-3'. Cells were plated in 6-well dishes and allowed to grow to ~70% confluency. Transfection of the gene specific siRNA plus a control siRNA (Ambion) was performed using the transmessenger transfection reagent (Ambion) according to the manufacturers protocol. 48 hours post-transfection, cells were lysed and western analysis was performed as described.

FIGURE LEGENDS

Figure 1. Over-expression of Bcl-3 inhibits DNA damage induced apoptosis **(A)** Expression of Bcl-3 in MCF-7, MCF-7B, Karpas 299 and HT-1080 cells. Western blots of extracts from indicated cell lines were probed with antibodies against Bcl-3 and β-tubulin. **(B)** MCF-7B cells are protected against DNA damage-induced apoptosis. MCF-7 and MCF-7B cells were left untreated, or treated with 40 J/m^2 UV-C or 10 μg/ml cisplatin as indicated. 18 hrs following treatment apoptosis was measured by flow cytometric analysis of Annexin-V staining.

Figure 2. Bcl-3 over-expression inhibits DNA damage-induced p53 activity. **(A)** UV-induced p53 protein levels are reduced in MCF-7B cells. MCF-7 and MCF-7B cells were treated with 40 J/m^2 UV-C for the indicated times and western analysis was performed on whole cell extracts using antibodies against Bcl-3, p53 and β-tubulin. **(B)**Transient expression of Bcl-3 inhibits p53 transcriptional activity. MCF-7 cells were transfected with 50 ng of pg-13-luciferase and 5 ng renilla luciferase plus 100 ng of pCMV-flag-Bcl-3 and pCMV-flag-p53 where indicated. Total DNA content was brought up to 255 ng with pCMV-flag vector. Firefly luciferase activity was measured and normalized to renilla luciferase. Values represent fold increase over basal activity (lane 1). **(C)** DNA damage-induced expression of p53 target genes is lost in MCF-7B cells. MCF-7 and MCF-7B cells were treated with 40 J/m2 UV-C for the indicated times and relative expression of p21, Noxa and Puma was measured by quantitative real-time PCR. Expression levels were normalized to expression of GUS and the values represent the fold increase or decrease relative to untreated MCF-7 cells (lane 1).

Figure 3. Loss of Bcl-3 leads to increased levels of p53 following DNA damage and sensitivity to DNA damage-induced apoptosis. **(A)** Knockdown of Bcl-3 results in an increase of UV-induced p53 protein. HT-1080 cells were transfected with a control siRNA or an siRNA targeting Bcl-3. 48 hrs following the transfection, cells were treated with 40 J/m^2 UV-C for the indicated timepoints. Western analysis was performed on whole cell extracts using antibodies against Bcl-3, p53 and β-tubulin. **(B)** Bcl-3 null MEFs have increased p53 levels following UV treatment. Wild-type and Bcl-3 deficient mouse embryonic fibroblasts were treated with 40 J/m2 UV-C for the indicated timepoints. Western analysis was performed on whole cell extracts using antibodies against p53 and β-tubulin. **(C)** Bcl-3 null MEFs are sensitive to UV-induced apoptosis. Bcl-3+/+ and Bcl-3-/- MEFs were left untreated, or treated with 40 J/m^2 UV-C as indicated. 18 hrs following treatment apoptosis was measured by flow cytometric analysis of Annexin-V staining.

Figure 4. Over-expression of Bcl-3 leads to an increase in Hdm-2 expression. **(A)** MCF-7B cells have higher basal levels of Hdm-2 protein. Whole cell extracts were prepared from MCF-7 and MCF-7B cells and western analysis was performed using antibodies against Bcl-3, Hdm-2 and β-tubulin. **(B)** MCF-7B cells have higher basal levels of Hdm-2 RNA. Real-time quantitative PCR was performed on cDNA prepared from MCF-7 and MCF-7B cells using primers specific for Bcl-3 and Hdm-2. Expression levels are normalized to expression of Gus and values represent fold difference relative to MCF-7 (lane 1) for each gene tested. **(C)** Transient over-expression of Bcl-3 leads to an increase in Hdm-2 levels in U-2OS cells. U-2OS cells were transfected with 0-200 ng of pCMV-flag-Bcl-3 in 50 ng increments. Total DNA content was brought to 200 ng with pCMV-flag vector. 48 hrs following transfection, western

analysis was performed on whole cell extracts using antibodies against Bcl-3, Hdm-2 and β-tubulin. **(D)** Bcl-3 is present at the Hdm-2 promoter at higher levels in MCF-7B cells. MCF-7 and MCF-7B cells were treated with 40 J/m2 UV-C for either 0 or 4 hours and Chromatin immunoprecipitation was performed using antibodies specific for p53, Bcl-3 or no antibody. Real-time quantitative PCR was performed on precipitated DNA using primers specific for the p2 promoter region of the Hdm-2 gene. Values are normalized against the input DNA and are represented as percent of input for each given sample.

Figure 5. Loss of Bcl-3 leads to a decrease in basal and DNA damage-inducible Hdm2 expression. **(A)** Knockdown of Bcl-3 in human cancer cells leads to loss of Hdm-2 expression. MCF-7 and KARPAS 299 cells were transfected with either control siRNAs or siRNAs specific for Bcl-3. 48 hours following transfection, western analysis was performed on whole cell extracts using antibodies specific for Bcl-3, Hdm-2 and β-tubulin. **(B)** DNA damage fails to induce Mdm-2 RNA in Bcl-3 deficient fibroblasts. Wild type and Bcl-3-null MEFs were treated with either 40 J/m2 UV-C or 10 μg/ml cisplatin for the indicated times and Mdm-2 gene expression was measured by quantitative real-time PCR. Expression levels were normalized to expression of GAPDH and the values represent the fold increase or decrease relative to untreated wild-type MEFs (lane 1). **(C)** DNA damage fails to induce Mdm-2 protein in Bcl-3 deficient fibroblasts. Wild type and Bcl-3-null MEFs were treated with 40 J/m2 UV-C for the indicated times and western blots were performed on whole cell extracts using antibodies specific for Mdm-2 and β-tubulin. **(D)** Knockdown of Bcl-3 impairs the ability of DNA damage to induce Hdm-2 in HT-1080 cells. HT-1080 cells were transfected with either control siRNAs or siRNAs specific for Bcl-3. 48 hours following transfection, cells were treated with 40 J/m^2 UV-C for the

indicated times and western analysis was performed on whole cell extracts using antibodies specific for Bcl-3, Hdm-2 and β-tubulin.

Figure 6. Disruption of the p53-Hdm2 interaction rescues the effects of Bcl-3 over-expression. **(A)** Disruption of the p53-Hdm-2 interaction restores the ability of UV to induce p53 in MCF-7B cells. MCF-7 and MCF-7B cells were pretreated for 30 minutes with either DMSO or 2μM RITA then treated with 40 J/m^2 UV-C for the indicated times. Whole cell extracts were prepared and subjected to western blot analysis using antibodies specific for p53 and β-tubulin. **(B)** Disruption of the p53-Hdm-2 interaction restores the ability of UV to induce apoptosis in MCF-7B cells. MCF-7B cells were left untreated, treated with 40 J/m^2 UV-C or treated with 40 J/m^2 UV-C plus 2 μM RITA. 18 hrs following treatment, apoptosis was measured by flow cytometric analysis of Annexin-V staining.